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A Simple, Fluorescent Method to Internally Label Platelets Suitable for Physiological Measurements

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Current methods for studying platelet survival in vivo are limited by the use of radioisotopes, with their inherent safety and regulatory concerns, systemic drug administrations that produce biochemical modifications of platelet functions, or external labeling techniques, which may produce artifacts due to surface modifications. For these reasons, we sought to develop a simple, nonisotopic method for labeling platelets internally, thereby producing platelets more likely to have in vivo properties equivalent to native cells. Murine platelets in protein-free buffer were fluorescently labeled internally by incubation with 2.5 μM 5-chloromethyl fluorescein diacetate (CMFDA), and without washing, were injected into mice for platelet survival studies. CMFDA-labeled platelets were unactivated, as shown by minimal P-selectin expression. When tested in vitro for function by aggregometry, the response of CMFDA-labeled platelets to collagen and thrombin was identical to that of unlabeled platelets. Flow cytometric analysis demonstrated that CMFDA platelets were an intensely stained, unimodal population that was completely separated from unlabeled platelets. The mean half-life of labeled platelets in the murine circulation was 37.5 ± 4.5 hr (±1 SD), and the mean survival time was 3.1–3.3 days (n = 24), similar to results reported using ^{51}Cr and ^{111}In. No evidence of in vivo transfer of dye from labeled platelets to unlabeled cells was observed. CMFDA produces a population of platelets that are nonradioactively, internally labeled with a highly fluorescent, stable product. The labeled platelets function equivalently to native platelets, as demonstrated by immunocytometry and aggregometry, and importantly, in vivo, by normal platelet survival. Am. J. Hematol. 56:17–25, 1997. © 1997 Wiley-Liss, Inc.

Key words: platelets; platelet lifespan; flow cytometry; CMFDA

INTRODUCTION

Measurement of platelet lifespan can discriminate between the mechanisms that produce thrombocytopenia, i.e., decreased production, peripheral destruction, or sequestration of platelets. Platelet lifespan is determined by labeling platelets by some methodology, and then monitoring their survival in the circulation over time. The ideal procedure would employ a label that was detectable with a high level of sensitivity, was stable over the lifespan of the platelet, and did not alter the normal functional and survival characteristics of the cells. All of the methods that have been described to date for labeling platelets have limitations. Radioisotopic methods utilizing ^{51}Cr or ^{111}In have been widely used [1–7]. However, these techniques have the disadvantage of quantifying radioactivity in a given volume of blood, and thus variations in the amounts of label per cell or inclusion of isotope not associated with cells of interest can affect the results. Also, there exists the possibility of internal radiation damage to cells, as well as additional safety, disposal, and regulatory concerns.

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Techniques involving nonisotopic modifications of some function of the entire platelet population, in vivo, also have been utilized to study platelet survival. Aspirin inactivates the cyclooxygenase pathway in platelets, with subsequent inhibition of the peroxidation of platelet lipids that is normally induced by various agents, e.g., thrombin. The recovery of lipid peroxidation function is then measured [8]. The administration of monoamine oxidase (MAO)-inhibiting drugs has been found to irreversibly inhibit platelet MAO, and thus the emergence of a population of platelets with MAO activity can be monitored [9]. In these methods, the recovery of a specific function was used as an indicator of platelet production, and thereby a potential measure of platelet lifespan, albeit an indirect one. However, some studies have reported a delay in recovery of normal function following administration of aspirin, because of the effect of aspirin on megakaryocytes [10–13]. The additional drawback of exposing individuals to these drugs with potential deleterious, systemic side-effects has limited their use.

Nonisotopic methods that involve surface labeling also have been reported. PKH2 (Zynaxis Cell Sciences, Inc., Malvern, PA), a fluorescent lipophilic chromophore that distributes into the lipids of the cell membrane, has been used to label many types of cells, e.g., in the study of red blood cell survival [14]. However, platelets are highly prone to aggregate after PKH2 labeling [14], rendering them suboptimal for survival studies. Biotinylation of platelets or whole blood ex vivo [15–17], or biotinylation in vivo [18–22], also have been used to study platelet survival. Ex vivo labeling of isolated platelets [16] requires manipulation of cells before reinfusion, and these techniques require multiple steps to fluorescently label each posttransfusion sample for flow cytometric analysis. In vivo labeling with biotin not only has the capability to label all circulating cells, but also may label precursor cells in bone marrow. Therefore, it has been suggested that new platelets generated from megakaryocytes may be detectably biotinylated [19,20]. This could potentially result in artificially long estimates of platelet lifespan. Additionally, an inherent drawback of these methods is that they involve alteration of the platelet surface, and therefore may modify platelet interactions with other platelets and endothelial surfaces. Finally, the elution of any surface probe must be considered.

5-chloromethylfluorescein diacetate (CMFDA) is a probe that freely passes through cell membranes. It is a colorless, nonfluorescent compound that contains a reactive chloromethyl group. Once inside a cell, the chloromethyl group reacts with intracellular thiols, transforming the probe into a cell-associated dye-thiol adduct. After cytosolic esterases cleave off the acetate groups of CMFDA, a brightly fluorescent product is generated. Excess unconjugated reagent diffuses passively into the extracellular medium.

CMFDA has been used to internally label rabbit platelets for in vitro adherence assays [23]. We now describe the use of CMFDA to label mouse platelets that are suitable for in vivo physiological studies, including platelet survival. The multiple advantages of this method are: 1) it is nonisotopic; 2) fluorescence is bright and stable over time; 3) the labeling is internal; and 4) minimal manipulations of the platelets are required.

MATERIALS AND METHODS

Reagents

CMFDA (5-chloromethylfluorescein diacetate) (Molecular Probes, Eugene, OR) was prepared as 2.5 mM stock in anhydrous DMSO (Sigma Chemical Co., St. Louis, MO). Anticoagulant stock solution (CEPT) contained the following: trisodium citrate (130 mM), disodium EDTA (10 mM), theophylline (100 mM), and PGI_2 (2 μg/ml). Buffered saline glucose citrate (BSGC), pH 6.8, consisted of: NaCl (116 mM), trisodium citrate (13.6 mM), Na_2HPO_4-$7H_2O$ (8.6 mM), KH_2PO_4 (1.6 mM), disodium EDTA (0.9 mM), glucose (11.1 mM), and PGI_2 (1 μg/ml) (all from Sigma Chemical Co.).

Preparation of Platelets for Labeling

Female Swiss Webster (SW) mice, 27–30 g (Simonsen Laboratories, Gilroy, CA), were used for these studies. Mice were housed in an American Association for Accreditation of Laboratory Animal Care-approved facility in filter cages, and fed standard rodent chow and tap water ad libitum. In conducting research using animals, the investigators adhered to the *Guide for the Care and Use of Laboratory Animals* prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH publication no. 86-23, revised 1985). All experimental protocols were approved by the Committee for Animal Experimentation of the Veterans Affairs Medical Center, San Francisco. Platelets were obtained for labeling from whole blood collected via cardiac puncture from mice anesthetized with methoxyflurane vapor (Metofane; Pitman-Moore, Inc., Mundelein, IL). Cardiac blood (1–1.5 ml) was drawn through a 25-gauge needle into a 3-ml plastic syringe containing 0.5 ml of CEPT anticoagulant diluted 1:1 (v/v) in BSGC. Five milliliters of pooled, fresh anticoagulated blood were diluted to 7 ml with BSGC and centrifuged at 600*g* for 3 min (22°C), and the platelet-rich plasma (PRP) was then collected. The residual blood sample was then rediluted to 7 ml with BSGC and recentrifuged to recover a second diluted PRP fraction, that was pooled with the first. Centrifugation of the PRP at 1,300*g* for 10 min (22°C) produced a platelet pellet that was suspended in BSGC to a final concentration of approximately 5×10^8 platelets/ml. The platelet concentrations of these samples were determined

with an electrical impedence counter (Model Z_H; Coulter Electronics, Hialeah, FL).

Platelet Labeling

Optimization of dye concentration. CMFDA, at final concentrations of 1, 2.5, 5, or 10 μM, was used to label platelets suspended in BSGC, which were then incubated in the dark for 45 min at 22°C. After incubation, the cells were centrifuged at 1,300*g* for 10 min (22°C), resuspended in fresh BSGC to the starting volume, and left in the dark for 30 min (22°C) to allow any excess unconjugated dye to diffuse out of the platelets, as recommended by the manufacturer. The platelets then were pelleted and resuspended in fresh BSGC to 5×10^8 cells/ml.

Effect of plasma on staining. CMFDA, at a concentration of 5 μM, was incubated in the dark with either PRP or platelets pelleted from PRP and resuspended in BSGC. After incubation, platelets were washed as described above.

Labeling for in vitro and in vivo studies. Platelets in BSGC were incubated in the dark with 2.5 μM CMFDA for 45 min (this concentration was selected on the basis of results from the previous optimization experiments). For the aggregation studies performed in vitro, platelets were centrifuged at 1,300*g* for 10 min (22°C) after incubation and resuspended in Tyrode's salt solution at a concentration of 3×10^8/ml. For platelet survival studies, approximately 2–2.5 $\times 10^8$ labeled platelets were directly injected i.v. into mice without further washing or additional concentration. In preliminary experiments, higher concentrations of CMFDA (5 and 10 μM) also were evaluated for determination of platelet survival.

Flow Cytometry

Flow cytometry was performed using a FACScan® (Becton-Dickinson, Inc., San Jose, CA), equipped with a 488-nm argon laser light source, a 530/30-nm band-pass filter in the FL1 emission path, and a 585/42-nm band-pass filter in the FL2 emission path. Cleaved CMFDA has spectral properties similar to fluorescein, and was detected in FL1. For selected experiments, a phycoerythrin (PE)-conjugate was used to determine P-selectin expression, which was detected in FL2. Platelets were distinguished from other cells and debris in whole-blood samples by their characteristic forward and side scatter. Labeled platelets were identified by using unstained platelets as a negative control to set a marker encompassing >99% of platelets. Platelets with fluorescence above this threshold were classified as labeled platelets.

Detection of P-selectin

The level of activation of CMFDA-labeled platelets was monitored by the expression of P-selectin on their surface. Platelets were resuspended in BSGC without PGI_2 for incubation in the dark with or without 2.5 μM CMFDA for 45 min at room temperature. PGI_2 was omitted to permit thrombin activation, and thus, these conditions maximized the potential for activation. After staining, 125-μl aliquots of the platelet suspensions were incubated for 10 min at 37°C with or without 1 U human thrombin and 10% (final concentration) of tissue culture supernatant containing rat monoclonal antibody RB40.34 against murine P-selectin (final total volume, 1 ml). RB40.34 was a generous gift from Dr. Dietmar Vestweber (Institute for Cell Biology, Wilhelms Universität, Münster, Germany). An equal volume of 0.6% formaldehyde then was added to each tube, and samples were incubated for 10 min at room temperature. After fixation, 8 ml of BSGC containing 0.1% bovine serum albumin (BSA), pH 7.4, were added and the samples were centrifuged at 1,300*g* for 10 min. The pellets were resuspended in 1 ml of BSGC with 0.1% BSA. Aliquots of approximately 10^6 platelets were then incubated at room temperature for 15 min with 1 μg of goat anti-rat IgG-PE (Caltag Laboratories, Burlingame, CA). Each sample was diluted to 1 ml with isotonic saline and analyzed by flow cytometry. Platelets in BSGC not exposed to CMFDA or RB40.34 but incubated with the goat anti-rat IgG-PE served as the negative control for P-selectin expression.

Platelet Aggregometry

The response of washed unlabeled and CMFDA-labeled platelets to human thrombin or calf skin collagen (both from Sigma) was assessed by conventional aggregometry, as described previously [24]. Aggregation studies were performed using platelets suspended in Tyrode's salt solution at a final concentration of 3×10^8 cells/ml. The baseline for minimal light transmission was set at the optical density of platelets in suspension; Tyrode's salt solution alone served as the standard for 100% light transmission. Platelet aggregation was detected as an increase in light transmission after the addition of thrombin or collagen. The lag phase was defined as the time interval between the addition of an agonist and the onset of aggregation.

Platelet Survival

Recipient mice were weighed and baseline blood samples were obtained from the retroorbital venous plexus, with the use of 70 μl heparinized EDTA-coated glass capillary tubes (Drummond Scientific Co., Broomall, PA). Platelet counts were determined from whole blood diluted 1:3 (v/v) in isotonic saline solution (Hematall, Fisher Scientific Co., Pittsburgh, PA), and analyzed with an automated flow cytometric whole-blood counter (Technicon H-1 System, Technicon Instruments, Tarrytown, NY), as previously described [25].

Aliquots of the labeled platelet suspensions (approximately 2–2.5 $\times 10^8$ platelets) were injected via a 27-

gauge needle and a 1-ml tuberculin syringe into a lateral tail vein of SW mice, after thermal dilation of the veins. After infusion of labeled platelets, blood samples were obtained from the retroorbital venous plexus at 2, 4, and 6 hr and then approximately every 12 hr for the next 4 days. Infusion of labeled platelets was not associated with evident toxicity or change in behavior of the recipient mice.

Percentages of labeled platelets in each whole-blood sample were determined by analysis of 50,000 platelets. Survival curves were constructed by designating the maximum number of circulating labeled platelets (as determined at either 2, 4, or 6 hr) as 100%, and expressing the sequential results as a percent of the maximum. The circulating half-life ($T_{1/2}$) was obtained from the survival curve. The survival time was estimated using either the multiple-hit model (gamma function) [15,26] or the best-fit estimate, derived from the use of both linear and exponential sum of squares calculations to describe the survival data. Dr. George Dale (University of Oklahoma Health Sciences Center, Oklahoma City, OK) generously provided the computer program for the multiple-hit analyses, and Dr. Sherrill Slichter (Puget Sound Blood Center, Seattle, WA) generously provided the program for the best-fit sum of squares analyses.

The total maximum number of circulating labeled platelets was calculated by the formula:

$$\text{Maximum \% circulating labeled platelets} \times \text{Total number of circulating platelets}$$

where the ''Maximum % circulating labeled platelets'' was determined by flow cytometry at 2, 4, or 6 hr, and ''Total number of circulating platelets'' was calculated by multiplying the platelet count/ml by the blood volume, estimated as 6% of body weight [7]. Percent post-transfusion recovery (R) was expressed as:

$$\frac{\text{Total maximum number of circulating labeled platelets}}{\text{Total number of transfused labeled platelets}} \times 100 = \%\text{R}.$$

The effect of storage conditions on the stability of platelets in the blood samples was examined. One group of samples was diluted immediately in 1 ml isotonic saline, and another group of the same samples was kept as whole blood, and similarly diluted just prior to analysis. Cell size, fluorescence, and percentage of labeled platelets in the samples were monitored daily for reproducibility over a period of 5 days, during which time both groups were stored at room temperature in the dark.

RESULTS

Optimization of Staining Conditions

There was a direct relationship between concentration of CMFDA and staining intensity. A concentration of 2.5 μM was the minimum required to produce an intensely stained population of platelets completely separated from the unlabeled population (Fig. 1). Platelets were observed by light microscopy and appeared discoid without pseudopods. The effect of plasma on labeling was examined by incubating PRP or washed platelets with 5 μM CMFDA. The staining intensity was greater in platelets labeled in plasma-free buffer than in platelets labeled in PRP (Fig. 2).

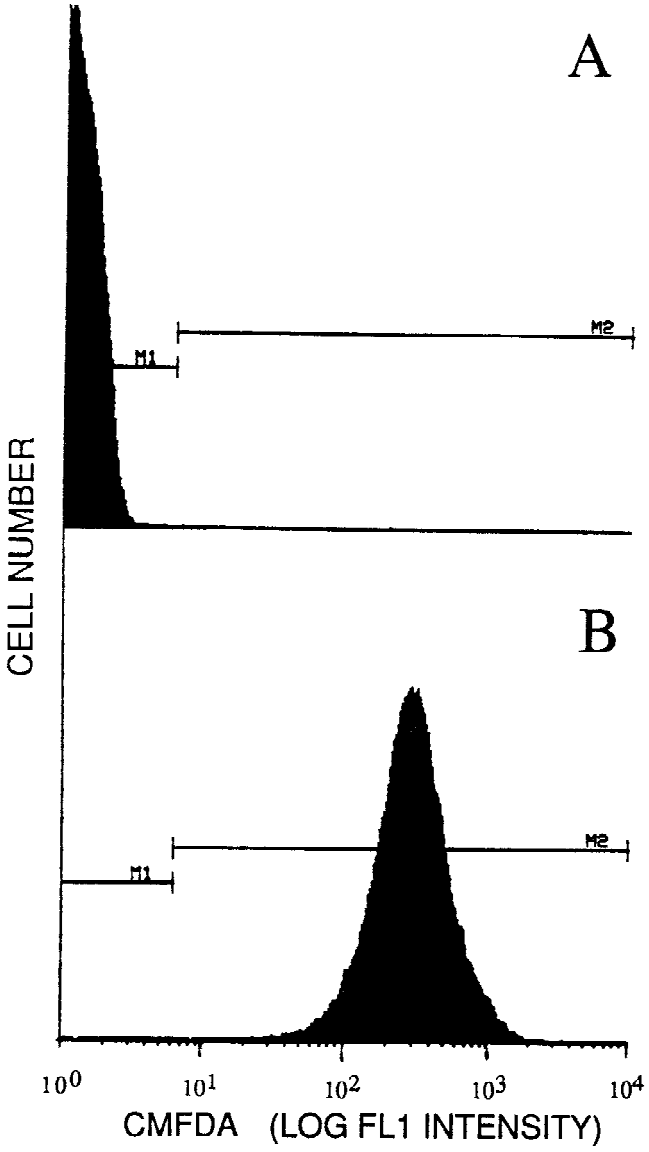

Fig. 1. Fluorescence intensities of unlabeled and CMFDA-labeled platelets. FL1 fluorescence intensity was determined by flow cytometry for washed unlabeled platelets (A), or washed platelets labeled with 2.5 μM CMFDA (B). Fifty thousand events were analyzed for each sample. The M1 marker encompasses >99% of unlabeled platelets, and indicates the level above which platelets were classified as labeled. Note that CMFDA-labeled platelets appeared as a discrete, unimodal population that was well-separated from unlabeled cells (>99% of platelets were beyond M1).

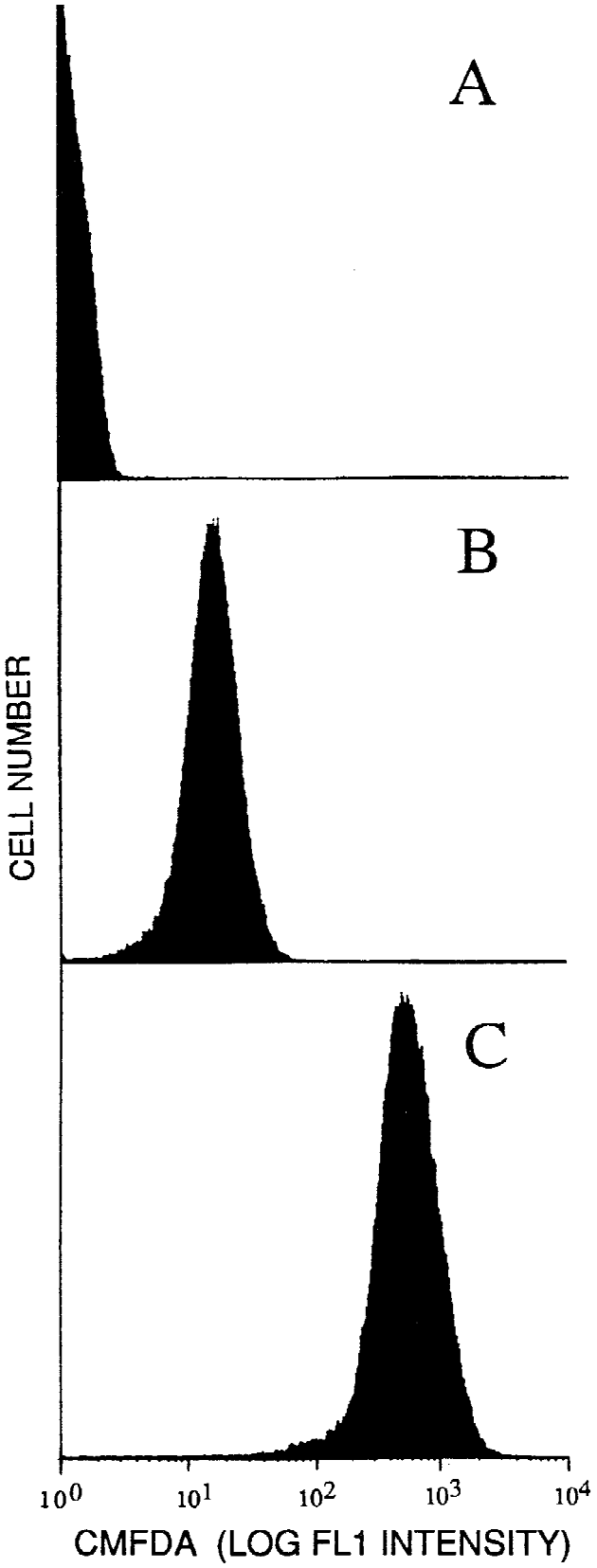

Fig. 2. Relative fluorescence intensity of platelets stained in the presence or absence of plasma. A: Log FL1 intensity of unlabeled platelets. B: Log FL1 intensity of PRP stained with 5 μM CMFDA. C: Log FL1 intensity of platelets in plasma-free BSGC stained with 5 μM CMFDA, which yielded the brightest unimodal population.

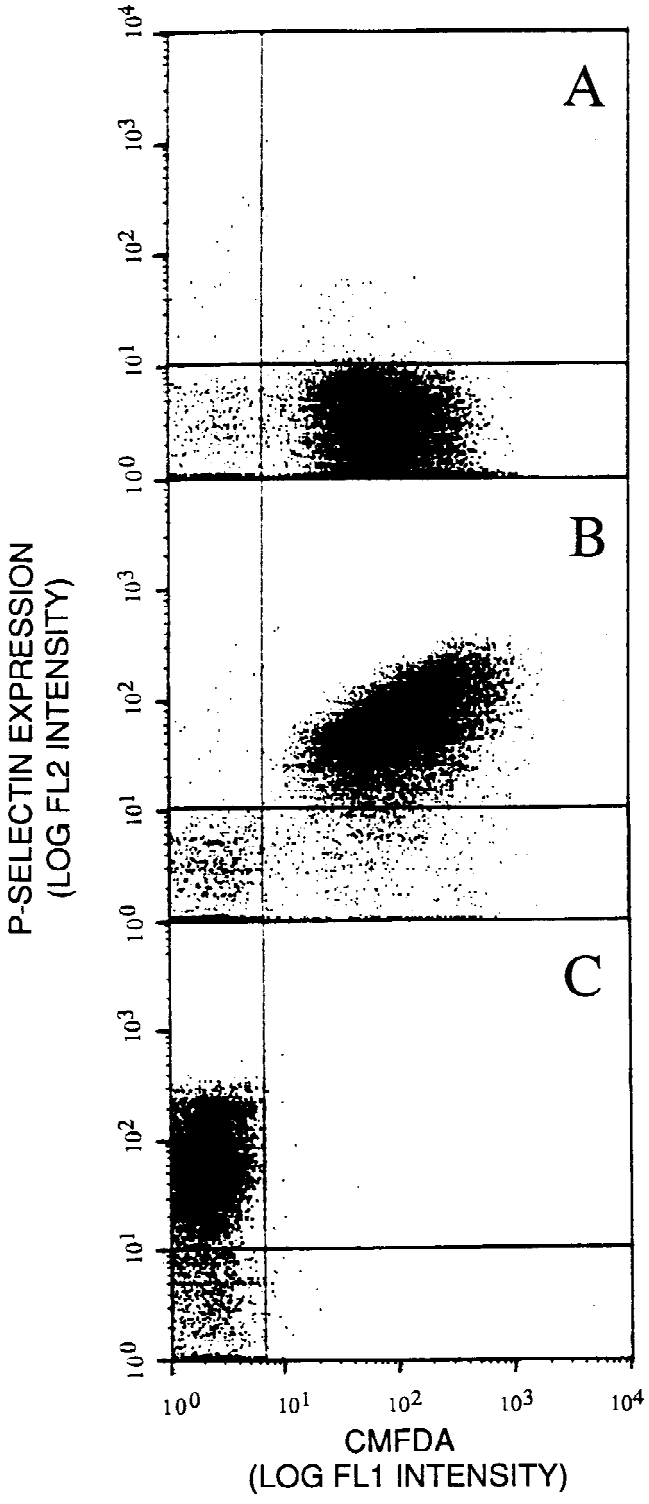

Fig. 3. P-selectin expression on platelets. Platelets suspended in BSGC without PGI_2 were analyzed for P-selectin expression on their surface. Levels of P-selectin (FL2) and CMFDA (FL1) are shown. A–C: Unstained cells are shown in the lower left quadrant. After labeling with 2.5 μM CMFDA, <1% of platelets were positive for P-selectin (upper right quadrant, A). When labeled platelets were activated by incubation with 1 U/ml thrombin, >90% of platelets expressed P-selectin (upper right quadrant, B), a level comparable to that observed with unlabeled platelets activated under the same conditions (upper left quadrant, C).

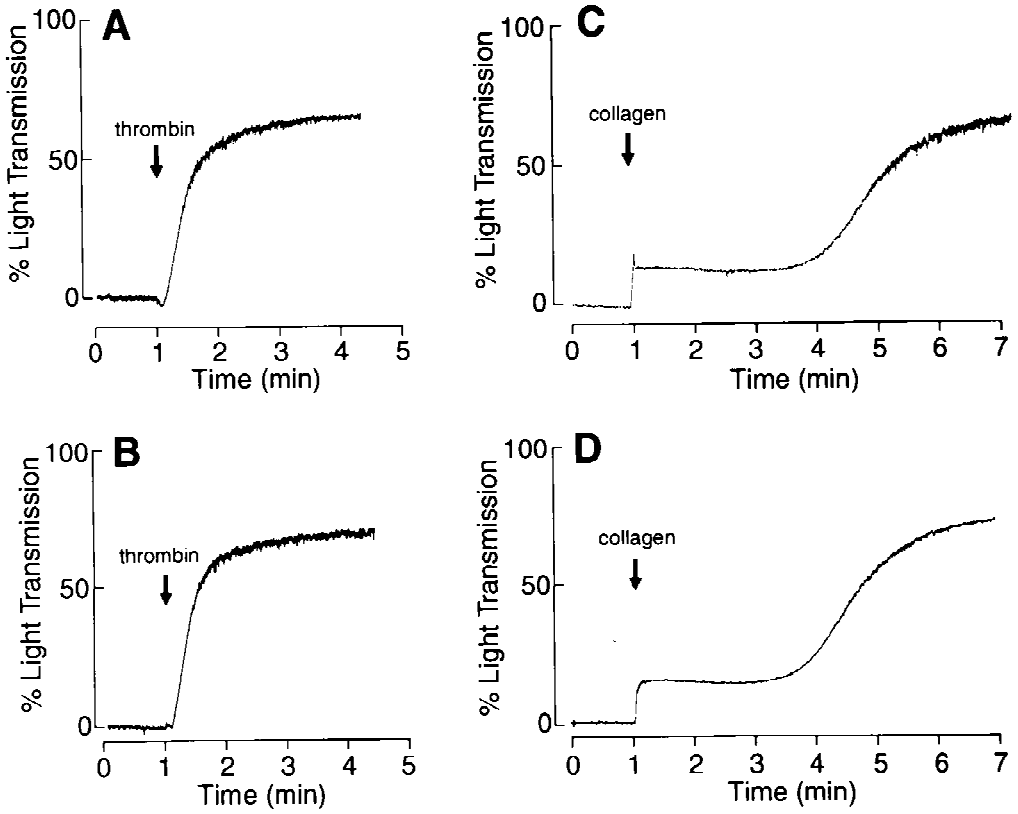

Fig. 4. Aggregation response of unlabeled and CMFDA-labeled mouse platelets. A: Unlabeled platelets and human thrombin (0.1 U/ml). B: CMFDA-labeled platelets and human thrombin (0.1 U/ml). C: Unlabeled platelets and calf-skin collagen (200 μg/ml). D: CMFDA-labeled platelets and calf-skin collagen (200 μg/ml). Arrows indicate time at which agonist was added to platelets. Results of a representative experiment are shown.

To establish that circulating unlabeled blood cells did not inadvertantly become labeled in vivo, after injection of CMFDA ex vivo-labeled platelets, three experiments were performed. Animals were injected i.v. with either 1) 0.4 ml of supernatant solution (approximately the volume of platelet suspension usually injected), obtained after platelets were labeled with 2.5 μM CMFDA for 45 min, and from which the platelets had been removed, 2) 2 μl of stock solution (2.5 mM) of CMFDA, or 3) platelets that had been labeled with 5 μM CMFDA, washed as previously described, and lysed by sonication for 30 sec. Serial samples were obtained as early as 1 hr after injections, and at various times for 18–21 hr, and analyzed by flow cytometry. No labeled platelets or other blood cells were detected at any time in these experiments (data not shown). The animals remained well.

Determination of P-selectin Expression

Platelets labeled with 2.5 μM CMFDA were monitored for surface expression of P-selectin, an α-granule membrane protein detectable on the cell surface only after secretion from activated platelets. After incubation with CMFDA, <1% of CMFDA-labeled platelets were positive for P-selectin (Fig. 3A), a percentage comparable to that seen with the negative, unlabeled control (not shown). After labeled platelets were activated with 1 U/ml human thrombin, >90% of these cells expressed P-selectin (Fig. 3B), a level comparable to that of unlabeled platelets activated under the same conditions (Fig. 3C).

In Vitro Platelet Function Studies

Thrombin-induced expression of P-selectin on CMFDA-labeled platelets suggested that these platelets were physiologically intact, i.e., capable of responding normally to platelet agonists. To examine this further, the aggregation response of washed unlabeled and labeled platelets to thrombin and collagen was assessed. Thrombin (0.1 U/ml) induced prompt, maximal, and irreversible platelet aggregation in both unlabeled and labeled platelets (Fig. 4A,B). Addition of 200 μg/ml collagen resulted in a characteristic lag phase [24], followed by irreversible aggregation (Fig. 4C,D). Thus, for both agonists, CMFDA-labeled platelets responded identically to unlabeled platelets.

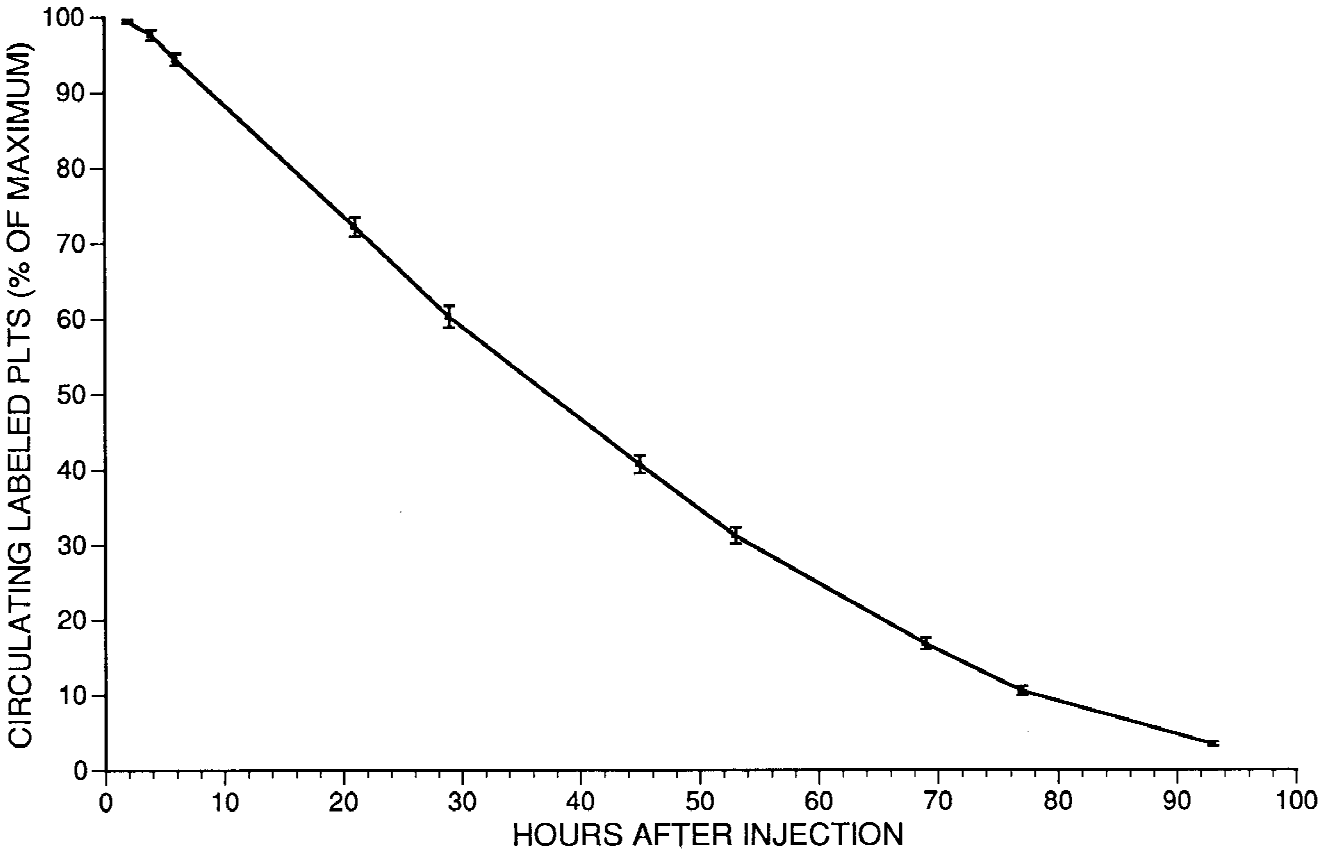

Fig. 5. Survival of CMFDA-labeled platelets in the circulation of normal SW mice. Platelets in BSGC were labeled with 2.5 μM CMFDA for 45 min in the dark at room temperature. Aliquots of approximately 2–2.5 × 10^8 platelets were injected into the tail veins of normal SW mice, and blood samples were obtained for analysis at the indicated time points. The maximum percent of circulating labeled platelets in each animal, as determined at 2, 4, or 6 hr, was designated as 100%. Mean values of the percent of maximum circulating labeled platelets (±1 SE) at multiple time points after injection were obtained from 24 mice. $T_{1/2}$ = 37.5 ± 4.5 hr (mean ± 1 SD).

Sample Stability

To determine if blood samples obtained from mice previously injected with CMFDA-labeled platelets were stable or had to be analyzed immediately, several characteristics of 10 samples were monitored. Forward scatter (FSC) is directly related to cell size. The FSC channel numbers of the labeled and unlabeled cells stored either diluted or as whole blood were compared. The FSC (size) of the labeled and unlabeled cells were indistinguishable under each storage condition for at least 2 days, indicating that no platelet swelling or aggregation had occurred. The fluorescence intensity of the platelets was virtually unchanged over 4 days for whole blood, and only slightly decreased in the diluted sample group, indicating excellent retention of intracellular fluorescence during storage (data not shown). The proportion of labeled platelets in the stored samples remained constant for 24 hr, but then decreased by 25% after 48 hr, and by 56% after 72 hr, irrespective of storage condition.

Platelet Recovery and Survival

Survival of platelets labeled with 2.5 μM CMFDA in the circulation was determined by serial bleeding of normal recipient mice over a period of 93 hr, and by analysis of the samples within 20 hr of collection. The mean platelet count of the recipient mice was 1.356 ± 0.224 × 10^9/ml (±1 SD). The mean maximal percent of circulating labeled platelets, determined at 2, 4, or 6 hr, was 7.95% ± 1.13% (±1 SD). The mean percent recovery of labeled platelets in the circulation was 66.7% ± 10.5% (±1 SD) (n = 24). The average $T_{1/2}$ was 37.5 ± 4.5 hr (±1 SD) (n = 24) (Fig. 5). The mean survival time, as estimated by multiple-hit (gamma function) analysis, was 3.1 ± 0.4 days (±1 SD), and by best-fit analysis, 3.3 ± 0.3 days (±1 SD). Preliminary data suggested that platelet survival was shortened when platelets were labeled with higher concentrations (e.g., 5 or 10 μM) of CMFDA.

The transfused platelets maintained a uniform level of fluorescence intensity during their circulation, indicating

excellent retention of the fluorescent product (Fig. 6). Labeled platelets remained a unimodal population, and were easily distinguished from unlabeled cells at all time points. The sizes of the unlabeled and labeled platelet populations remained constant, indicating that neither cell disintegration nor clumping had occurred (data not shown).

DISCUSSION

Previous methods for labeling platelets for survival studies have been hampered by the use of radioactive isotopes with their inherent problems, external labeling with nonisotopes that alter the platelet surface and could yield artifacts, or metabolic labels that require administration of drugs and provide only an indirect measurement of platelet lifespan. In view of these limitations, we developed a simple, nonradioactive method for labeling platelets that remain suitable for physiological measurements.

Labeling with CMFDA is rapid, and because of our observation that excess unconjugated dye is not taken up by cells in the circulation, the often troublesome necessity for platelet washing after staining is eliminated, making this method simple, and less likely to produce platelet damage. The minimal manipulations resulted in platelets that exhibited no evidence of activation, as determined by light microscopy and the lack of P-selectin expression on their surface. Because platelets are intensely stained and are analyzed in a flow cytometer on a cell-by-cell basis, very small samples are required; serial measurements can be performed for lifespan studies without significantly perturbing the total blood volume or altering the regulation of platelet production [27].

CMFDA labeling is internal, in contrast to surface labeling that can alter membranes and predispose platelets to spontaneous aggregation or activation, thereby resulting in inaccurate quantification of the number of platelets injected and generating falsely shortened lifespan data. Platelet labeling with CMFDA is stable. The intensity of CMFDA labeling was shown to remain constant throughout the lifespan of the platelets, suggesting excellent retention. We found no evidence of CMFDA transfer from cell to cell. In addition, blood samples were stable when kept either undiluted, or diluted in isotonic saline, for 24 hr; therefore, samples need not be analyzed immediately,

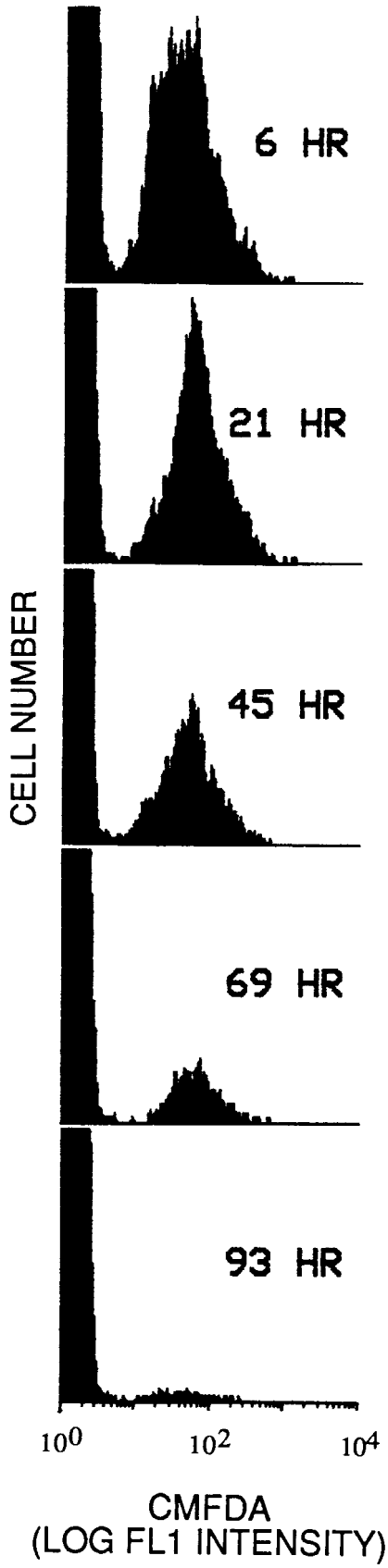

Fig. 6. Serial measurements of fluorescence intensity of labeled platelets. Platelets were labeled with 2.5 μM CMFDA for 45 min, and injected into SW mice. Whole-blood samples were collected at the time points indicated, and the CMFDA content of platelets (FL1 fluorescence intensity) was serially measured for 93 hr. A series of histograms from a single mouse, representative of the 24 independent studies that constitute Figure 5, is shown.

but can be accumulated if necessary, and run when convenient.

Using this technique, the response of labeled platelets to platelet agonists, as assessed by platelet aggregometry, remained normal. Platelet survival studies utilizing CMFDA-labeled platelets injected into mice demonstrated 66.7% recovery and produced survival curves exhibiting a $T_{1/2}$ of 37.5 hr, in good agreement with previous ^{51}Cr studies which demonstrated platelet recoveries of between 60–70% and half-lives of 39–44 hr [3,7]. The survival time of 3.1–3.3 days is in good agreement with values of 2.1–4 days obtained for survival of mouse platelets labeled with ^{111}In [1,5].

Therefore, we conclude that CMFDA provides an improved method for labeling platelets for survival studies. It is potentially useful for human as well as animal studies, if systemic toxicity in humans is absent. Additionally, the described properties render this method potentially useful for numerous other applications, e.g., platelet-binding studies, in vitro and in vivo; serial measurements of stably labeled circulating platelets, from which subpopulations could be identified; or studies in which the uniformly labeled platelets could be recovered by cell-sorting and then further characterized.

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